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Do glucagonomas always produce glucagon?

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ABSTRACT

Pancreatic islet α -cell tumours that overexpress proglucagon are typically associated with the glucagonoma syndrome, a rare disease entity characterised by necrolytic migratory erythema, impaired glucose tolerance, thromboembolic complications and psychiatric disturbances. Paraneoplastic phenomena associated with enteric overexpression of proglucagon-derived peptides are less well recognized and include gastrointestinal dysfunction and hyperinsulinaemic hypoglycaemia. The diverse clinical manifestations associated with glucagon-expressing tumours can be explained, in part, by the repertoire of tumourally secreted peptides liberated through differential post-translational processing of tumour-derived proglucagon. Proglucagon-expressing tumours may be divided into two broad biochemical subtypes defined by either secretion of glucagon or GLP-1, GLP-2 and the glucagon-containing peptides, glicentin and oxyntomodulin, due to an islet α -cell or enteroendocrine L-cell pattern of proglucagon processing, respectively. In the current review we provide an updated overview of the clinical presentation of proglucagon-expressing tumours in relation to known physiological actions of proglucagon-derived peptides and suggest that detailed biochemical characterisation of the peptide repertoire secreted from these tumours may provide new opportunities for diagnosis and clinical management.

KEY WORDS: Neuroendocrine tumours; NETs; hyperglucagonemia; GLP-1; proglucagon

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BOX 1. LEARNINGS POINTS

- Proglucagon-derived peptides exhibit a diverse range of biological activities including critical roles in regulation of glucose and amino acid metabolism, and possibly also affect energy homeostasis as well as cardiovascular and gastrointestinal function.
- Clinical manifestations of proglucagon-expressing tumours exhibit marked phenotypic variation possibly due to heterogeneity of their secreted peptide repertoire.
- Specific and precise biochemical assessment of individuals with proglucagon-expressing tumours may provide opportunities for improved diagnosis and clinical management.

INTRODUCTION

Gastroenteropancreatic neuroendocrine tumours are rare with an annual incidence rate of less than 10 per million population [1]. These tumours can be subdivided into non-functioning and functioning, the latter referring to tumours capable of secreting peptide hormones or biogenic amines into the circulation in a dysregulated manner to produce defined tumour syndromes with varying clinical manifestations.

Glucagonomas arise mostly from pathological transformation of pancreatic islet α -cells that synthesise and secrete proglucagon-derived peptides (Figure 1). These rare tumours have an annual incidence of 1 per 20-40 million population [2]; however autopsy studies have reported the incidence of islet cell tumours with glucagon expressing cells to approximate 1% suggesting that a number of tumours are undiagnosed and/or associated with sub-clinical disease [3]. The vast majority of proglucagon-expressing tumours are sporadic (80%) with the remainder associated with Multiple Endocrine Neoplasia-type 1 (MEN1), an inherited tumour predisposition syndrome, or Mahvash disease, an extremely rare cause of familial pancreatic α -cell hyperplasia and

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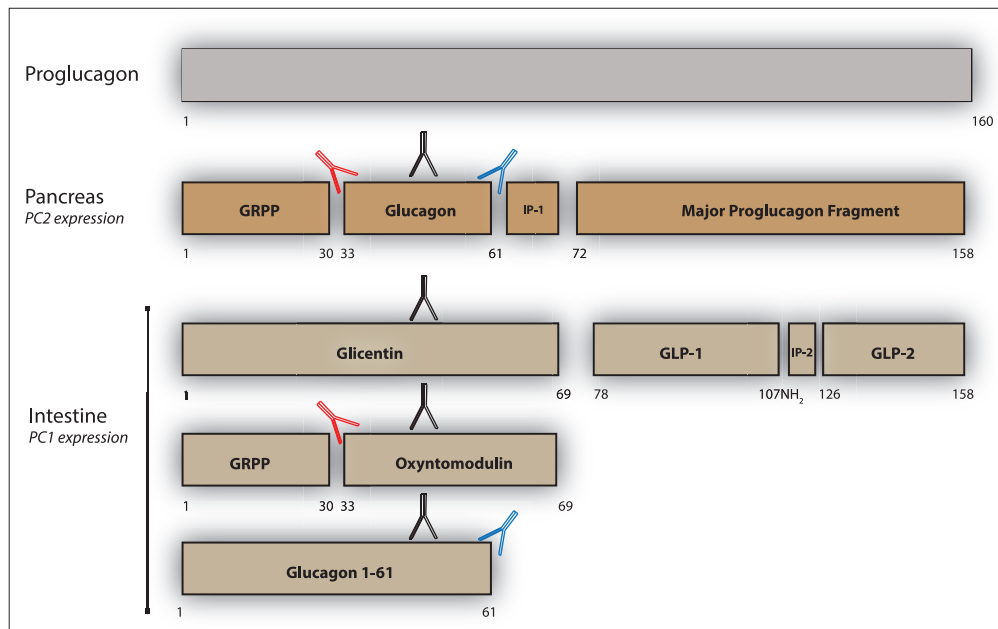


FIGURE 1. Tissue specific processing of proglucagon. In the pancreatic α -cell, proglucagon is processed by PC2 activity into GRPP, glucagon, major proglucagon fragment (MPGF). In the L-cells, proglucagon is processed by PC1/3 into GLP-1 (7-36NH₂) GLP-2 and glicentin. By further PC1/3 activity, glicentin can then be further cleaved into glicentin related pancreatic polypeptide (GRPP) and oxyntomodulin. N-terminal elongated glucagon has also been reported and is termed intestinal glucagon or enteroglucagon. Black arrowheads refer to side-viewing proglucagon antibodies, red arrowheads refer to N-terminal glucagon/oxyntomodulin antibodies and blue arrowheads refer to C-terminal glucagon antibodies. Glucagon measurement should therefore be carried out using, preferably a sandwich ELISA employing N and C-terminal glucagon antibodies. Assay only using black, blue or red antibodies (as depicted here) may over or underestimate glucagon levels due to cross-reactivity with other proglucagon molecules such as glicentin, oxyntomodulin or enteroglucagon 1-61.

glucagonoma due to inactivating mutations in the glucagon receptor (*GCGR*) gene [4].

CLINICAL HETEROGENEITY OF GLUCAGONOMAS

Only a subset of glucagonomas manifest clinical symptoms resulting in a spectrum of disease; ranging from an asymptomatic patient to one with hallmarks of the glucagonoma syndrome. The classic features associated with glucagonomas include a characteristic dermatitis termed necrolytic migratory erythema (NME), weight loss, impaired glucose tolerance or diabetes mellitus, painful glossitis, stomatitis, diarrhoea, thromboembolic complications and psychiatric disease [5]. More recently, scattered reports have also described glucagonomas presenting with acute heart failure and dilated cardiomyopathy that are reversible following therapeutic normalization of circulating glucagon levels [6, 7]. Less commonly recognized are the paraneoplastic phenomena associated with tumoral secretion of proglucagon-derived GLP-1 and GLP-2 which may present with hyperinsulinaemic hypoglycaemia and marked gastrointestinal dysfunction (refractory constipation, reduced motility, gross structural abnormalities of the small intestine), respectively [8-10] (Figure 2). The diagnosis of glucagonoma is established by demonstrating hyperglucagonaemia (>500 mg/mL).

However, in the absence of clinical symptoms and signs the diagnosis should be reconsidered given elevated glucagon levels are also found in association with cirrhosis, renal failure, pancreatitis, diabetes mellitus, prolonged fasting and familial hyperglucagonaemia [2,11] (thus not as high as the glucagonomas).

NME is a presenting feature in 70% of patients with glucagonoma. It is usually widely distributed with the groin, perineum and distal extremities most commonly affected. Clinically, NME is characterized by raised erythematous lesions with fragile bullae that break down and shed into crusted areas of hyperpigmentation. Histological examination of biopsied skin demonstrates small bullae and necrosis of the upper epidermis. Although the aetiology of NME remains unclear, histological similarities with pellagra (nicotinic acid deficiency) and acrodermatitis enteropathica (zinc deficiency) suggest NME is a consequence of nutrient deficiency. A number of case reports have also described iatrogenic NME following intravenous glucagon infusion suggesting that PGDPs may have direct effects on the skin [12]. Interestingly, despite profound hyperglucagonaemia, patients with Mahvash disease do not develop NME thereby suggesting that intact glucagon-receptor signalling is required for NME pathogenesis [4,13]. NME invariably improves with normalization of glucagon levels through either surgical reduction of tumour burden or medical treatment with somatostatin analogues [14].

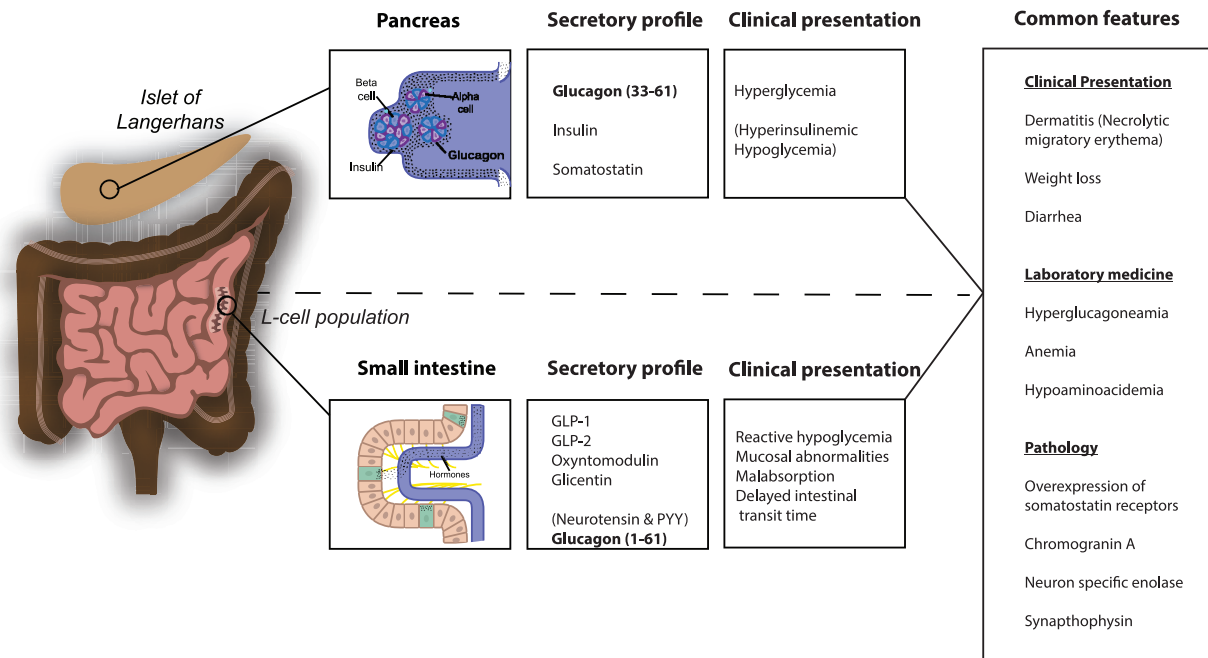


FIGURE 2. The molecular heterogeneity of glucagonomas results in differential clinical phenotype. As illustrated patients suffering with glucagonomas shares common features (right panel) however and clinical important they may depending on the molecular processing of proglucagon also.

Weight loss is the most common presenting feature of proglucagon-expressing tumours reflecting important roles for proglucagon-derived peptides in the regulation of energy homeostasis [15,16]. GLP-1 and glucagon act independently to potentially inhibit food intake through activation of central satiety centres. In addition, glucagon modulates energy balance through stimulation of energy expenditure possibly via the sympathetic nervous system to activate thermogenesis in brown adipose tissue (BAT). Whilst such an effect of glucagon has been repeatedly demonstrated in experimental animal models the importance of BAT activation in human energy balance remains uncertain. Nevertheless, GLP-1 and glucagon both promote negative energy balance which may result in profound weight loss in pathological states, such as in the glucagonoma syndrome, when these peptide hormones may be secreted at high levels.

Glucagon is the archetypical counter-regulatory hormone, which is normally released in response to hypoglycaemia to promote hepatic glucose production but is also essential for maintenance of euglycaemia in the fasting state. Despite these actions, diabetes mellitus is not an inevitable consequence of the glucagonoma syndrome although the presence of diabetes mellitus shown to correlate directly with plasma glucagon levels [17]. Glucagon strongly stimulates insulin secretion, which of course has opposite effects on glycaemia, which may explain that not all patients with high glucagon levels have diabetes. In these cases, diabetes may develop secondary to impairment of pancreatic endocrine function because of the tumour. It is unclear, however, whether patients with mildly

elevated glucagon levels have impaired glucose intolerance in the absence of the full blown glucagonoma syndrome. Moreover, it is not known whether patients with sub-clinical disease are at increased risk of thromboembolic, gastrointestinal or cardiovascular complications is not known and further study is required to determine whether such patients would benefit from prophylactic anticoagulation and/or regular echocardiographic assessment of cardiac function.

Finally, similar with other pancreatic neuroendocrine tumours, glucagonomas may co-secrete a multitude of hormones including insulin, PYY, pancreatic polypeptide, ACTH, PTH, somatostatin and PTHrP, thereby adding further complexity to the clinical phenotype. These endocrine disturbances which may delay correct diagnosis [18].

MOLECULAR HETEROGENEITY OF GLUCAGONOMAS

The variable clinical phenotypes seen with proglucagon-expressing tumours may be due, in part, to the specific secreted peptide repertoire derived from proglucagon in that tumour. The physiology of proglucagon derived peptides have been comprehensively reviewed elsewhere [19-24]. Here, we provide a brief overview of the processing and physiological actions of proglucagon-derived peptide hormone as they relate to proglucagon-expressing tumours.

As illustrated in Figure 1, post-translational proglucagon processing differs between pancreatic islet α -cells and intestinal enteroendocrine L-cells. The biologically most important

product of α -cell proglucagon processing is presumably glucagon, a 29 amino acid hormone which is formed from proglucagon together with the probably inactive processing products, glicentin-related pancreatic polypeptide (GRPP) (corresponding to proglucagon 1-30) and Major Proglucagon Fragment (MPGF) (corresponding to proglucagon 72-158), as a result of the actions of prohormone convertase 2 (PC2) in these cells [25]. Intestinal proglucagon-expressing enteroendocrine L cells, distributed throughout the small and large intestine, *do not*, at least normally and in healthy subjects, secrete glucagon (33-61) but rather liberate glicentin, oxyntomodulin, GLP-1 and GLP-2 derived from proglucagon following tissue-specific processing by prohormone convertase 1/3 (PC1/3). The intact, bioactive GLP-1 moieties, GLP-1(7-36) amide (~90 % in humans [26]) and GLP-1(7-37) (~10 %, but more so e.g. in rats and pigs [27]), are released upon appropriate stimulation of the L cells and mediate their biological actions through activation of the GLP-1 receptor expressed in pancreatic β -cells and several extrapancreatic tissues including brain, heart, kidney and peripheral and enteric nervous systems.

Hyperglucagonemia may disrupt glucose homeostasis through opposing mechanisms as either a diabetogen to promote hyperglycemia and/or promoting post-prandial hypoglycemia through stimulation of insulin secretion [28]. In glucagonoma, the diabetogenic effects of glucagon may predominate as up to 80% of patients have a diagnosis of diabetes mellitus at presentation [29,30]. Whilst pancreatic derived glucagon appears important in both health and disease, it is intriguing to note that hyperglucagonaemia is also found in patients who have undergone pancreatectomy, suggesting that the intestinal L-cell may be a potential source of circulating fully processed glucagon (sequence 33-61) in some disease states [31].

The observations that GLP-1, an incretin hormone, has glucose-lowering properties and attenuates glucagon secretion have stimulated pharmaceutical development of several GLP-1 analogues, such as exenatide and liraglutide, for the treatment of type 2 diabetes. Notably, when used in isolation these therapies are associated with low risk of hypoglycaemia. Proglucagon expressing tumours that secrete GLP-1 have been described and appear to result in hypoglycaemia [8]. Roberts *et al* reported GLP-1 secretion from a metastatic pancreatic NET resulting in pancreatic β -cell hyperplasia and hyperinsulinism [32] whereas Todd and colleagues described an ovarian NET co-secreting GLP-1 and somatostatin presenting with both reactive hypoglycaemia and diabetes [33]. Interestingly, however, symptomatic hypoglycaemia is not a presenting feature of all GLP-1 secreting NETs (Brubaker *et al*, Byrne *et al*) which may be due to aberrant proglucagon processing by the tumour to produce biologically inactive but immunoreactive GLP-1 moieties [9,34]. When tumorally secreted, GLP-1

mediated hypoglycaemia is suspected, biochemical confirmation of elevated fasting levels of bioactive GLP-1 during hypoglycaemia is required. Further evidence may be provided by the absence of insulin expression in tumour biopsies and improvement of hypoglycaemia following normalization of GLP-1 levels and/or presence of pancreatic islet cell hyperplasia which has been observed when GLP-1 secreting tumours are present [32,35].

In humans, intestinal enteroendocrine L-cells synthesise glicentin which may be processed further by PC1/3 to liberate GRPP and oxyntomodulin, a peptide hormone with the ability to increase insulin and glucagon secretion *ex-vivo* and *in-vivo* [36,37] as well as inhibit appetite and promote weight loss in overweight subjects [38]. Glicentin has no known effect on glucose or appetite regulation but early reports proposed that this peptide hormone may possess intestinotropic properties following descriptions of patients with giant duodenal villi and small bowel hypertrophy due to glicentin-secreting proglucagonomas [39-41]. Advances in immunoassays capable of measuring specific proglucagon-derived peptides in concert with improved understanding of the roles of PDGPs in gastrointestinal physiology suggest these early observations were not solely due to elevated glicentin and more likely reflect the intestinotropic actions of GLP-2. In response to gut injury, GLP-2 promotes nutrient absorption through activation of crypt cell proliferation and expansion of small bowel epithelium and perhaps more importantly inhibits epithelial apoptosis. Therapeutically, a synthetic GLP-2 analogue (teduglutide) is approved for treatment of short bowel syndrome and intestinal failure [42]. GLP-1 is also intestinotropic but appears less efficacious than GLP-2 and is a more potent inhibitor of gastric motility when compared with GLP-2 [21].

Initial recognition of an association between the development of intestinal villous hyperplasia and proglucagon derived peptides was provided by clinical description of a patient with features of the glucagonoma syndrome and giant duodenal villi due to an enteroglucagon producing tumour of the kidney [41]. Subsequent reports have confirmed these early findings and identified tumoral co-secretion of GLP-2 and GLP-1 as the peptide hormones responsible for intestinal villous hyperplasia associated with refractory constipation, reduced absorptive capacity, delayed gastric emptying, nausea and vomiting [2,9,43,44]. We recently described the first case of hyperinsulinaemic hypoglycaemia and marked gastrointestinal dysfunction due to a GLP-1 and GLP-2 secreting tumour (Challis *et al* unpublished observations). Here, the use of immunoassays directed against specific PDGPs in combination with size-exclusion chromatography allowed for detailed biochemical characterization of the secreted peptide repertoire [40,45].

PDGPs influence cardiovascular biology through a diverse range of actions. Glucagon, for example, has well recognized chronotropic actions on the heart and therapeutically is

considered the drug of choice for treating bradycardia and hypotension due to beta-blocker poisoning. Whilst current understanding of glucagon action in the human heart is limited, recent data derived from murine models of reduced glucagon signalling in cardiomyocytes suggest that following myocardial infarction, glucagon negatively influences survival, cardiac hypertrophy and left ventricular remodelling [46]. In contrast, several studies have demonstrated that GLP-1 has cardio-protective actions mediated via several independent and poorly understood mechanisms some of which involve may involve GLP-1R activation in atrial cardiomyocytes. However, pharmacological strategies that promote increased GLP-1 levels in subjects with type 2 diabetes mellitus have not consistently demonstrated improved cardiovascular outcomes in large randomized controlled trials [48,49]. In patients with glucagonoma, single cases of reversible acute left ventricular dysfunction [6] and dilated cardiomyopathy [7] in association with hyperglucagonaemia have been described, but overall cardiac decompensation is a rare consequence of pathological levels of glucagon. As questioned previously, whether patients with sub-clinical disease and mild elevations in glucagon are at risk of clinically significant cardiac dysfunction is not known and we would advocate echocardiographic surveillance for such patients.

The secretory output of glucagonomas depends on the tumoural expression of PC1/3 or PC2. Although likely rare, given the limited number of reported cases, there is some evidence to suggest that glucagonomas may undergo a transition from a α -cell processing profile (PC2-mediated processing) to one that resembles the enteroendocrine L-cell (PC1/3-mediated processing [32]. IL-6 [50] and metabolic stress, such as hyperglycaemia [51], have been shown to induce PC1/3 expression in pancreatic α -cells and in the context of malignancy circulating pro-inflammatory cytokines may alter the tumour phenotype. Clinically, this may present with GLP-1 mediated hyperinsulinaemic hypoglycaemia in an individual with a previous history of diabetes or features of the glucagonoma syndrome. Indeed, several such cases have been reported in the medical literature [32].

BIOCHEMICAL ASSESSMENT OF HYPERGLUCAGONAEMIA

Measurement of glucagon remains a key element in the diagnosis of glucagonomas. However, the differentiation between tumoural hypersecretion of pancreatic glucagon and glucagon-like immunoreactivity (Figure 1 and 2) is challenging and may affect the subsequent choice of treatment strategy [45]. How can this be challenging? There may, at least, be two explanations both related to the biochemical assessment of pancreatic glucagon. First, immuno-based detection methods

(be it radioimmunoassays or ELISAs) may lack specificity for pancreatic glucagon (33-61) as the L-cell derived peptides glicentin and oxyntomodulin contains the glucagon sequence (see Figure 1) and, in addition, that GLP-1 and GLP-2 shares amino acid similarities. Several glucagon kits claimed to be specific have been demonstrated not to be with some even detecting other proglucagon derived peptides but not pancreatic glucagon [52]. Secondly, the pancreatic specific glucagon kits employing C-terminal antibodies (this is the classic way of measuring glucagon [30]) may also react with N-terminal elongated forms [45] and thereby overestimating pancreas (33-61) specific glucagon.

FUTURE OUTLOOK FOR IMPROVED DIAGNOSTIC AND TREATMENT OF GLUCAGONOMAS

In summary, tumoural expression of proglucagon-derived peptides may result in a diverse spectrum of clinical phenotypes dependent on the specific secreted peptide repertoire. The advent of biochemical methods that quantify specific tumour-derived proglucagon-derived peptides has provided opportunities for detailed tumour phenotyping, biochemical sub-classification and improved understanding of the clinical manifestations associated with these rare tumours.

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DECLARATION OF INTEREST

The authors have declared that no conflict of interest exists.

REFERENCES

- [1] Kaltsas GA, Besser GM, Grossman AB. The Diagnosis and Medical Management of Advanced Neuroendocrine Tumors. *Endocrine Reviews*. 2004;25(3):458-511. <http://dx.doi.org/10.1210/er.2003-0014>.
- [2] Economopoulos P, Christopoulos C. Glucagonoma. *Annals of Gastroenterology* 2007.
- [3] Öberg K. Neuroendocrine gastro-enteropancreatic tumors – from eminence based to evidence-based medicine – A Scandinavian view. *Scandinavian journal of gastroenterology*. 2015;50(6):727-39. <http://dx.doi.org/10.3109/00365521.2015.1033001>.
- [4] Yu R, Ren S-G, Mirocha J. Glucagon receptor is required for long-term survival: A natural history study of the Mahvash disease in a murine model. *Endocrinología y Nutrición*. 2012;59(9):523-30. <http://dx.doi.org/10.1016/j.endonu.2012.06.006>.
- [5] Ito T, Igarashi H, Jensen RT. Pancreatic neuroendocrine tumors: Clinical features, diagnosis and medical treatment: *Advances. Best Practice & Research Clinical Gastroenterology* 26(6):737-53. <http://dx.doi.org/10.1016/j.bpg.2012.12.003>.

- [6] Zhang K, Lehner LJ, Praeger D, Baumann G, Knebel F, Quinkler M, et al. Glucagonoma-induced acute heart failure. *Endocrinology, Diabetes & Metabolism Case Reports*. 2014;2014:140061. <http://dx.doi.org/10.1530/edm-14-0061>.
- [7] Chang-Chretien K, Chew JT, Judge DP. Reversible dilated cardiomyopathy associated with glucagonoma. *Heart*. 2004;90(7):e44-e.
- [8] Guimarães M, Rodrigues P, Pereira SS, Nora M, Gonçalves G, Albrechtsen NW, et al. GLP1 and glucagon co-secreting pancreatic neuroendocrine tumor presenting as hypoglycemia after gastric bypass. *Endocrinology, Diabetes & Metabolism Case Reports*. 2015;2015:150049. <http://dx.doi.org/10.1530/edm-15-0049>.
- [9] Brubaker PL, Drucker DJ, Asa SL, Swallow C, Redston M, Greenberg GR. Prolonged Gastrointestinal Transit in a Patient with a Glucagon-Like Peptide (GLP)-1- and -2-Producing Neuroendocrine Tumor. *The Journal of Clinical Endocrinology & Metabolism*. 2002;87(7):3078-83. <http://dx.doi.org/10.1210/jcem.87.7.8584>.
- [10] Iglesias P, Diez JJ. Management of endocrine disease: a clinical update on tumor-induced hypoglycemia. *European journal of endocrinology/European Federation of Endocrine Societies*. 2014;170(4):R147-57.
- [11] van Beek AP, de Haas ER, van Vloten WA, Lips CJ, Roijers JF, Canninga-van Dijk MR. The glucagonoma syndrome and necrolytic migratory erythema: a clinical review. *European journal of endocrinology/European Federation of Endocrine Societies*. 2004;151(5):531-7. <http://dx.doi.org/10.1530/eje.0.1510531>.
- [12] Roy SM, Coughlin CC, Adzick NS, Yan AC, Rubin AI, Leon DDD. Iatrogenic Necrolytic Migratory Erythema in an Infant Receiving Glucagon Therapy for Congenital Hyperinsulinism. *Late-breaking Pediatric Endocrinology I2015*. p. LBT-028-LBT.
- [13] MB; L, VE; Y, R Y. Mahvash Disease: Pancreatic Neuroendocrine Tumor Syndrome Caused by Inactivating Glucagon Receptor Mutation. *J Mol Genet Med*. 2013;7(84).
- [14] Zhang M X, Shen Y, Hu ZH, Wu LM, Zheng SS. Clinical experience in diagnosis and treatment of glucagonoma syndrome. *Hepatobiliary Pancreat Dis Int*. 2004;3(3):473-5.
- [15] Ruttimann EB, Arnold M, Hillebrand JJ, Geary N, Langhans W. Intrameal hepatic portal and intraperitoneal infusions of glucagon-like peptide-1 reduce spontaneous meal size in the rat via different mechanisms. *Endocrinology*. 2009;150(3):1174-81. <http://dx.doi.org/10.1210/en.2008-1221>.
- [16] Holst JJ, Guldberg Madsen O, Knop J, Schmidt A. The effect of intra-portal and peripheral infusions of glucagon on insulin and glucose concentrations and glucose tolerance in normal man. *Diabetologia*. 1977;13(5):487-90. <http://dx.doi.org/10.1007/BF01234501>.
- [17] Wermers RA, Fatourech V, Wynne AG, Kvols LK, Lloyd RV. The glucagonoma syndrome. Clinical and pathologic features in 21 patients. *Medicine (Baltimore)*. 1996;75(2):53-63. <http://dx.doi.org/10.1097/00005792-199603000-00002>.
- [18] Ito T, Igarashi H, Jensen RT. Pancreatic neuroendocrine tumors: clinical features, diagnosis and medical treatment: advances. *Best practice & research Clinical gastroenterology*. 2012;26(6):737-53. <http://dx.doi.org/10.1016/j.bpg.2012.12.003>.
- [19] Wewer Albrechtsen NJ, Kuhre RE, Deacon CF, Holst JJ. Targeting the intestinal L-cell for obesity and type 2 diabetes treatment. *Expert Review of Endocrinology & Metabolism*. 2014;9(01):61-72. <http://dx.doi.org/10.1586/17446651.2014.862152>.
- [20] Holst JJ. Incretin hormones and the satiation signal. *International journal of obesity (2005)*. 2013;37(9):1161-8. <http://dx.doi.org/10.1038/ijo.2012.208>.
- [21] Holst JJ. Glucagon and glucagon-like peptides 1 and 2. *Results ProblCell Differ*. 2010;50:121-35. doi: 10.1007/400_2009_35:121-35.
- [22] Holst JJ. The Physiology of Glucagon-like Peptide 1. *Physiological Reviews*. 2007;87(4):1409-39. <http://dx.doi.org/10.1152/physrev.00034.2006>.
- [23] Pocai A. Action and therapeutic potential of oxyntomodulin. *Molecular Metabolism*. 2014;3(3):241-51. <http://dx.doi.org/10.1016/j.molmet.2013.12.001>.
- [24] Drucker DJ, Yusta B. Physiology and Pharmacology of the Enteroendocrine Hormone Glucagon-Like Peptide-2. *Annual Review of Physiology*. 2014;76(1):561-83. <http://dx.doi.org/10.1146/annurev-physiol-021113-170317>.
- [25] Holst JJ, Bersani M, Johnsen AH, Kofod H, Hartmann B, Orskov C. Proglucagon processing in porcine and human pancreas. *The Journal of biological chemistry*. 1994;269(29):18827-33.
- [26] Orskov C, Rabenhøj L, Wettergren A, Kofod H, Holst JJ. Tissue and plasma concentrations of amidated and glycine-extended glucagon-like peptide I in humans. *Diabetes*. 1994;43(4):535-9. <http://dx.doi.org/10.2337/diab.43.4.535>.
- [27] Kuhre RE, Albrechtsen NW, Windelov JA, Svendsen B, Hartmann B, Holst JJ. GLP-1 amidation efficiency along the length of the intestine in mice, rats and pigs and in GLP-1 secreting cell lines. *Peptides*. 2014;55:52-7. <http://dx.doi.org/10.1016/j.peptides.2014.01.020>.
- [28] Kawai K, Yokota C, Ohashi S, Watanabe Y, Yamashita K. Evidence that glucagon stimulates insulin secretion through its own receptor in rats. *Diabetologia*. 1995;38(3):274-6. <http://dx.doi.org/10.1007/BF00400630>.
- [29] Meier JJ, Ueberberg S, Korbas S, Schneider S. Diminished glucagon suppression after β -cell reduction is due to impaired α -cell function rather than an expansion of α -cell mass. *American Journal of Physiology - Endocrinology and Metabolism*. 2011;300(4):E717-E23. <http://dx.doi.org/10.1152/ajpendo.00315.2010>.
- [30] Wewer Albrechtsen N, Hartmann B, Veedfald S, Windeløv J, Plamboeck A, Bojsen-Møller K, et al. Hyperglucagonaemia analysed by glucagon sandwich ELISA: nonspecific interference or truly elevated levels? *Diabetologia*. 2014;1-8. <http://dx.doi.org/10.1007/s00125-014-3283-z>.
- [31] Holst JJ, Pedersen JH, Baldissera F, Stadil F. Circulating glucagon after total pancreatectomy in man. *Diabetologia*. 1983;25(5):396-9. <http://dx.doi.org/10.1007/BF00282517>.
- [32] Roberts RE, Zhao M, Whitelaw BC, Ramage J, Diaz-Cano S, Roux CWL, et al. GLP-1 and Glucagon Secretion from a Pancreatic Neuroendocrine Tumor Causing Diabetes and Hyperinsulinemic Hypoglycemia. *The Journal of Clinical Endocrinology & Metabolism*. 2012;97(9):3039-45. <http://dx.doi.org/10.1210/jc.2011-2005>.
- [33] Todd JF, Stanley SA, Roufosse CA, Bishop AE, Khoo B, Bloom SR, et al. A tumour that secretes glucagon-like peptide-1 and somatostatin in a patient with reactive hypoglycaemia and diabetes. *The Lancet*. 2003;361(9353):228-30. [http://dx.doi.org/10.1016/S0140-6736\(03\)12256-8](http://dx.doi.org/10.1016/S0140-6736(03)12256-8).
- [34] Byrne MM, McGregor GP, Barth P, Rothmund M, Göke B, Arnold R. Intestinal Proliferation and Delayed Intestinal Transit in a Patient with a GLP-1-, GLP-2- and PYY-Producing Neuroendocrine Carcinoma. *Digestion*. 2001;63(1):61-8. <http://dx.doi.org/10.1159/000051874>.
- [35] Patti M-E, Goldfine A. Hypoglycemia after Gastric Bypass: The Dark Side of GLP-1. *Gastroenterology*. 2014;146(3):605-8. <http://dx.doi.org/10.1053/j.gastro.2014.01.038>.
- [36] Baldissera FG, Holst JJ, Knuhtsen S, Hilsted L, Nielsen OV. Oxyntomodulin (glicentin-(33-69)): pharmacokinetics, binding to liver cell membranes, effects on isolated perfused pig pancreas, and secretion from isolated perfused lower small intestine of pigs. *RegulPept*. 1988;21(1-2):151-66. [http://dx.doi.org/10.1016/0167-0115\(88\)90099-7](http://dx.doi.org/10.1016/0167-0115(88)90099-7).
- [37] Schjoldager BT, Baldissera FG, Mortensen PE, Holst JJ, Christiansen J. Oxyntomodulin: a potential hormone from the distal gut. *Pharmacokinetics and effects on gastric acid and insulin secretion in man. EurJClinInvest*. 1988;18(5):499-503. <http://dx.doi.org/10.1111/j.1365-2362.1988.tb01046.x>.
- [38] Wynne K, Park AJ, Small CJ, Patterson M, Ellis SM, Murphy KG, et al. Subcutaneous oxyntomodulin reduces body weight in overweight and obese subjects: a double-blind, randomized, controlled trial. *Diabetes*. 2005;54(8):2390-5. <http://dx.doi.org/10.2337/diabetes.54.8.2390>.
- [39] Hashimoto N, Ohyanagi H, Kazuyuki S. Effect of glicentin on gut

- mucosal growth in rats with jejunal and ileal Thiry-vella fistulas. *Journal of Pediatric Surgery*. 2003;38(4):579-84.
<http://dx.doi.org/10.1053/jpsu.2003.50126>.
- [40] Bloom SR. An enteroglucagon tumour. *Gut*. 1972;13(7):520-3.
<http://dx.doi.org/10.1136/gut.13.7.520>.
- [41] Gleeson MH, Bloom SR, Polak JM, Henry K, Dowling RH. Endocrine tumour in kidney affecting small bowel structure, motility, and absorptive function. *Gut*. 1971;12(10):773-82.
<http://dx.doi.org/10.1136/gut.12.10.773>.
- [42] Jeppesen PB, Hartmann B, Thulesen J, Graff J, Lohmann J, Hansen BS, et al. Glucagon-like peptide 2 improves nutrient absorption and nutritional status in short-bowel patients with no colon. *Gastroenterology*. 2001;120(4):806-15.
<http://dx.doi.org/10.1053/gast.2001.22555>.
- [43] Jones B, Fishman EK, Bayless TM, Siegelman SS. Villous hypertrophy of the small bowel in a patient with glucagonoma. *J Comput Assist Tomogr*. 1983;7(2):334-7.
<http://dx.doi.org/10.1097/00004728-198304000-00027>.
- [44] Lax E, Leibovici V, Fields SI, Gordon RL. Neglected radiologic signs of the glucagonoma syndrome. *Diagnostic imaging in clinical medicine*. 1986;55(6):321-6.
- [45] Holst JJ. Molecular heterogeneity of glucagon in normal subjects and in patients with glucagon-producing tumours. *Diabetologia*. 1983;24(5):359-65.
- [46] Ali S, Ussher JR, Baggio LL, Kabir MG, Charron MJ, Ilkayeva O, et al. Cardiomyocyte glucagon receptor signaling modulates outcomes in mice with experimental myocardial infarction. *Molecular Metabolism*. 2015;4(2):132-43.
<http://dx.doi.org/10.1016/j.molmet.2014.11.005>.
- [47] Ussher JR, Drucker DJ. Cardiovascular Actions of Incretin-Based Therapies. *Circulation Research*. 2014;114(11):1788-803.
<http://dx.doi.org/10.1161/CIRCRESAHA.114.301958>.
- [48] Green JB, Bethel MA, Armstrong PW, Buse JB, Engel SS, Garg J, et al. Effect of Sitagliptin on Cardiovascular Outcomes in Type 2 Diabetes. *New England Journal of Medicine*. 2015;373(3):232-42.
<http://dx.doi.org/10.1056/NEJMoa1501352>.
- [49] Scirica BM, Bhatt DL, Braunwald E, Steg PG, Davidson J, Hirshberg B, et al. Saxagliptin and Cardiovascular Outcomes in Patients with Type 2 Diabetes Mellitus. *New England Journal of Medicine*. 2013;369(14):1317-26.
<http://dx.doi.org/10.1056/NEJMoa1307684>.
- [50] Donath MY. Targeting inflammation in the treatment of type 2 diabetes: time to start. *Nat Rev Drug Discov*. 2014;13(6):465-76.
<http://dx.doi.org/10.1038/nrd4275>.
- [51] Ellingsgaard H, Hauselmann I, Schuler B, Habib AM, Baggio LL, Meier DT, et al. Interleukin-6 enhances insulin secretion by increasing glucagon-like peptide-1 secretion from L cells and alpha cells. *Nat Med*. 2011;17(11):1481-9.
<http://dx.doi.org/10.1038/nm.2513>.
- [52] Bak MJ, Albrechtsen NW, Pedersen J, Hartmann B, Christensen M, Vilsbøll T, et al. Specificity and sensitivity of commercially available assays for glucagon and oxyntomodulin measurement in humans. *European Journal of Endocrinology*. 2014;170(4):529-38.
<http://dx.doi.org/10.1530/EJE-13-0941>.